Mapping out starvation responses

What are the pathways that underlie the coordinated responses of an organism to well-fed and food-deprived states? A report in this issue of *Cell Metabolism* suggests that starvation functions via a muscarinic acetylcholine receptor to activate MAP kinase signaling in the pharyngeal muscle of *C. elegans* (You et al., 2006).

In their natural environments, most organisms are faced with limited food supplies; the ability of organisms to withstand food deprivation is therefore critical to survival (Gray et al., 2004; Wang et al., 2005). With the notable exception of people in affluent economic classes in recent history, fluctuating food sources also characterize the human experience. When food is scarce, reductions in energy expenditure must be balanced with strategies for escaping predation and maintaining food-seeking capacity. Thus, successful survival strategy for animals requires dynamic alterations of metabolism, physiology, and behavior in response to changing environmental conditions (Wang et al., 2005). Molecular mechanisms that mediate these responses are largely unknown. To uncover signaling cascades induced by starvation, You et al. (2006) investigated potential mechanisms in the *C. elegans* pharynx, the nematode's feeding organ. They showed that starvation activates acetylcholine signaling through a muscarinic receptor to phosphorylate a MAPK (mitogen-activated protein kinase) and that hyperactivation of the pathway is detrimental when *C. elegans* are starved.

In general, starvation induces a wide range of responses that include altered gene expression and biochemical activities, exit from the cell cycle, alternative developmental states (e.g., bacterial spores and biofilm, dauer formation in *C. elegans*), and altered physiological and behavioral responses (Gray et al., 2004; Wang et al., 2005). Additionally, food deprivation and poststarvation feeding elicit pronounced structural changes in size and functional capacity of organs in animals including humans. While these changes have been noted for many visceral organs, they are most pronounced for the gastrointestinal tract (Secor and Diamond, 1998; Wang et al., 2005). A remarkable example is the 2-fold increase in kidney and intestinal mucosal mass of Burmese pythons upon feeding (Secor and Diamond, 1998).

C. elegans provides an opportunity to dissect signaling mechanisms that mediate starvation responses with genetic, pharmacological, and biochemical tools. *C. elegans* feed through the powerful pumping action of the pharyngeal tube (Figure 1A) that results in bacterial ingestion, concentration, grinding, and forcing of the crushed suspension into the intestinal lumen (Avery and Shtonda, 2003). Under laboratory conditions, continuous pharyngeal pumping is observed in both well-fed and starved *C. elegans*; however, pumping rate is modulated by food availability. For healthy, young adult *C. elegans,* the pharyngeal pumping rate of starved animals is roughly one fourth that of animals fed on their laboratory diet of *E. coli*. Introduction of food to starved animals causes pumping rate to increase to levels beyond that of well-fed animals. Moreover, to maximize food consumption, *C. elegans* respond to increasing concentrations of bacteria by increasing their pumping rates. Finally, starved animals exhibit pumping increases in response to much lower concentrations of bacteria, which demonstrates that the starvation sensitizes *C. elegans* to nutrient levels (Avery and Horvitz, 1990).

Avery and colleagues had previously shown that acetylcholine regulates pharyngeal pumping through coordinated signaling of both ionotropic and muscarinic (G protein-coupled) acetylcholine receptors found on pharyngeal muscles (Steger and Avery, 2004). To identify molecular mechanisms that alter pharyngeal muscle function in response to starvation, You and colleagues (2006) took a candidate approach. A common response in eukaryotes to stresses including starvation is activation of MAPK signaling. Based on this hunch, the authors used a phospho-specific anti-MAPK antibody to show that starvation promotes phosphorylation of a pharyngeally expressed MAPK. Interestingly, a muscarinic receptor agonist similarly enhanced the phosphorylation of this pharyngeal MAPK while a muscarinic receptor antagonist blocked this starvation-induced phosphorylation.

Next, You and colleagues (2006) genetically identified the specific muscarinic receptor and downstream signaling components that mediate starvation induced activation of the pharyngeal MAPK reporter (Figure 1B). They took advantage of mutant *C. elegans* strains as well as a previous observation that loss of function *gpb-2* mutants are hypersensitive to muscarinic signaling. This is because *gpb-2* encodes for the β subunit of a heterotrimeric G protein and functions to negatively regulate signaling through Gqα. Treatment by the muscarinic agonist arecoline causes pharyngeal hypercontraction leading to lethality in *gpb-2* mutant animals. The authors showed that starvation mimics arecoline treatment of *gpb-2* mutant animals. They reasoned that taking out molecules that transduce the starvation signal should block pharyngeal MAPK phosphorylation and rescue the starvation hypersensitivity of *gpb-2* mutant animals. Using this strategy, the authors determined that starvation likely functions through *gar-3*, one of three muscarinic receptors encoded by the *C. elegans* genome, to activate Gqα, followed by activation of a novel protein kinase C that leads to phosphorylation of MAPK (You et al., 2006).

These discoveries lay the groundwork for investigating a number of interesting questions: Given that acetylcholine signaling is important for fast pumping in well-fed animals, how do starvation signals alter acetylcholine signaling? For example, is the neuronal source of acetylcholine different in starved animals? How do *C. elegans* sense and monitor environmental nutrient availability? Finally, an important but unanswered question is elucidation of the physiological roles of starvation-induced MAPK phosphorylation. The present study indicates that hyperactivation of the pharyngeal MAPK pathway alters pharyngeal morphology with detrimental effects. One possibility advanced by the authors is that phosphorylation of pharyngeal MAPK could

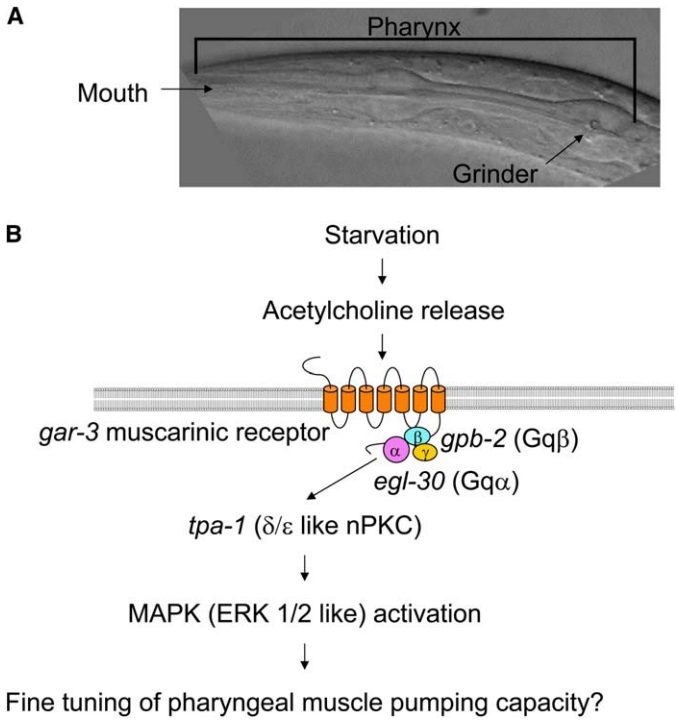

Figure 1. Identification of starvation responsive signaling cascades in the *C. elegans* pharyngeal muscle

A) A differential interference contrast image of a *C. elegans* pharynx. The bilobed pharynx is a self-contained organ with an autonomous nervous system, muscles, gland cells, and structural cells. The pumping action of the pharynx allows for food ingestion and is modulated by food availability. Starved animals display dramatically reduced pumping rates relative to well-fed animals. Image courtesy of Elisabeth Greer, UCSF.

B) Model for starvation-induced phosphorylation of pharyngeal MAPK. You and colleagues (2006) propose that starvation activates muscarinic receptor signaling to cause phosphorylation of pharyngeal MAPK. The signaling cascade includes the *gar-3* muscarinic receptor, *egl-30* Gqα, and a novel PKC with homology to mammalian δ/ε PKC. Loss-of-function mutations in Gqβ, encoded by *gpb-2*, promote signaling by removing a negative regulator of Gqα.

fine tune pumping rates such that starved animals maintain reduced pumping but are poised to increase pumping rate when food is encountered. Another likely possibility is that MAPK activation serves an energetic role by down-regulating energy utilization pathways of the pharyngeal muscle, one of the biggest muscles of *C. elegans*. Clues to the role of starvation induced MAPK activation may emerge from the identification of pharyngeal MAPK targets.

In mammals, muscarinic acetylcholine receptors regulate heart muscle and smooth muscle of the gastrointestinal tract, and MAPK (ERK) signaling activation downstream of muscarinic acetylcholine receptors has been widely noted. Moreover, there are intriguing but conflicting reports on the role M3 muscarinic receptor in growth rate and body weight of rodents (Matsui et al., 2004). It is therefore plausible that molecular mechanisms that mediate starvation responses of *C. elegans* pharyngeal muscle are conserved across phylogeny.

Managing a successful living strategy with intermittent bouts of food availability and other environmental perturbations is not an easy task. Along with pumping rate, food availability modulates *C. elegans* locomotory rate and foraging behavior (Sawin et al., 2000). Studies of have revealed remarkably complex regulatory mechanisms that impact these food seeking behaviors. In addition to acetylcholine, neurotransmitters such as glutamate, serotonin, and octapomine impact pumping rate. The latter two may also mediate signals related to the presence or absence of food (Rex et al., 2004). Serotonin and dopamine signaling impact locomotory rate (Sawin et al., 2000). Finally, a neuropeptide Y signaling pathway determines whether *C. elegans* display solitary or social feeding patterns (de Bono and Bargmann, 1998). The genetic and anatomical advantages of *C. elegans* can help reveal the molecular components of the complex pathways that allow animals to interact and respond to their environments.

Kaveh Ashrafi[1]
[1] Department of Physiology and
The UCSF Diabetes Center
University of California
San Francisco, Genentech Hall N-412C
San Francisco, California 94143

Selected reading

Avery, L., and Horvitz, H.R. (1990). J. Exp. Zool. *253*, 263–270.

Avery, L., and Shtonda, B.B. (2003). J. Exp. Biol. *206*, 2441–2457.

de Bono, M., and Bargmann, C.I. (1998). Cell *94*, 679–689.

Gray, J.V., Petsko, G.A., Johnston, G.C., Ringe, D., Singer, R.A., and Werner-Washburne, M. (2004). Microbiol. Mol. Biol. Rev. *68*, 187–206.

Matsui, M., Yamada, S., Oki, T., Manabe, T., Taketo, M.M., and Ehlert, F.J. (2004). Life Sci. *75*, 2971–2981.

Rex, E., Molitor, S.C., Hapiak, V., Xiao, H., Henderson, M., and Komuniecki, R. (2004). J. Neurochem. *91*, 1104–1115.

Sawin, E.R., Ranganathan, R., and Horvitz, H.R. (2000). Neuron *26*, 619–631.

Secor, S.M., and Diamond, J. (1998). Nature *395*, 659–662.

Steger, K.A., and Avery, L. (2004). Genetics *167*, 633–643.

Wang, T., Hung, C.C., and Randall, D.J. (2005). Annu. Rev. Physiol., in press. Published online October 19, 2005. 10.1146/annurev.physiol.68.040104.105739.

You, Y., Kim, J., Cobb, M., and Avery, L. (2006). Cell Metab. *3*, this issue, 237–245.

DOI 10.1016/j.cmet.2006.03.002